

DEVELOPMENT AND ACTIVATION OF ORGANELLES IN *PROTEA COMPACTA* EMBRYOS DURING GERMINATION

J. VAN STADEN AND M. G. GILLILAND

(Department of Botany, University of Natal, Pietermaritzburg)

ABSTRACT

The development of glyoxysomes, on which organelle activation in the meristematic root-tip of *Protea compacta* embryos depends, appears to be closely connected with the nuclear and membrane state of the cells.

UITTREKSEL

DIE ONTWIKKELING EN AKTIVERING VAN ORGANELLE IN *PROTEA COMPACTA* EMBRIOS GEDURENDE ONTKIEMING.

Die ontwikkeling van glioksosome, waarvan die aktivering van organelle in die meristemiese wortelpunt van *Protea compacta* afhanklik is, is waarskynlik nou gebonde aan die staat van die kerne en membrane in die betrokke selle.

INTRODUCTION

In an attempt to determine the primary requirements for germination of *Protea compacta* seed it was deemed necessary to investigate the ultrastructural and biochemical changes that occur in these seeds during the germination process. The ultrastructure of dry embryos from both viable and non-viable seed (Van Staden *et al.*, 1975a) and the digestion of food reserves during incubation (Van Staden *et al.*, 1975b) have been reported on. The present paper reports on the development and activation of organelles (or lack thereof) in these embryos.

MATERIAL AND METHODS

Seeds of *Protea compacta* R. Br. were incubated under favourable germination conditions (Brown and Van Staden, 1973) for 20 days. At regular intervals embryos were excised and prepared for electron microscopy as previously described (Van Staden *et al.*, 1975a).

OBSERVATIONS AND DISCUSSION

In the dry state, cells in the meristematic region of the root-tip of viable embryos contained apparently quiescent nuclei. The nucleolus was compact and consisted mainly of fibrillar material. Very little heterochromatin was present in the nucleoplasm. In contrast, nuclei in non-viable embryos contained extensive dense patches of chromatin (Van Staden *et al.*, 1975a). Proplastids,

mitochondria and tubular ER could be observed compressed between the protein and lipid bodies and near the periphery of the cells. Although ribosomes were present in the hyaloplasm it was apparent that protein metabolism and the organelles were in a very low state of activity.

After three days of incubation the nucleolar material in viable embryos increased considerably and became much less compact (Fig. 1). This was accompanied by the appearance of polysomes in the cytoplasm where the ribosomes were arranged in spirals, suggesting the presence of mRNA (Bonnett and Newcomb, 1965) (Fig. 2). During the first five days of incubation only fragments of tubular ER could be observed and mitochondria had few cristae (Fig. 3). Between five and ten days of incubation microbodies with a dense granular content and a single bounding membrane could be seen in intimate association with the lipid bodies. These were identified as glyoxysomes (Van Staden *et al.*, 1975b). After the appearance of these organelles there was a decrease in the number of lipid bodies and starch began to appear in the plastids, which had acquired a rudimentary prolamellar system as a result of budding from the inner membrane of the envelope (Fig. 4). Starch ultimately becomes the main food reserve of these cells.

The development of glyoxysomes, which are involved in lipid digestion via the glyoxylate pathway and thus in the generation of energy, is of primary importance for subsequent development of the embryo that ultimately results in radicle elongation. (Van Staden *et al.*, 1975c). In non-viable embryos where this development did not occur no organelle development could be observed.

Following the appearance of glyoxysomes the meristematic cells after 15 days develop an elaborate system of sheet-like rough ER, liberally studded with ribosomes (Fig. 5). Numerous apparently active mitochondria, dictyosomes (Fig. 6) and multi-vesicular bodies also appeared. These latter developments are probably concerned with cell elongation, as the radicles emerge shortly afterwards.

The available evidence suggests that there is some chromosome damage in the necrotic nuclei of the non-viable embryo, which affects the genome and hence protein metabolism (Van Staden *et al.*, 1975a). One of the results of this might be the inability to produce glyoxysomes making it impossible to generate sufficient energy for germination. It has been shown by Villiers (1973) that extensively damaged membrane systems, as occur in non-viable *P. compacta* embryos (Van Staden *et al.*, 1975a), have an inhibitory effect on germination.

ACKNOWLEDGEMENTS

The authors would like to thank the C.S.I.R., Pretoria, for financial support and the EM Unit of the University of Natal, Pietermaritzburg, for technical assistance.

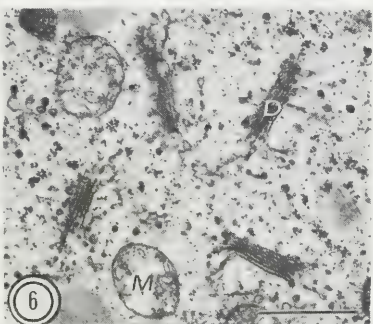
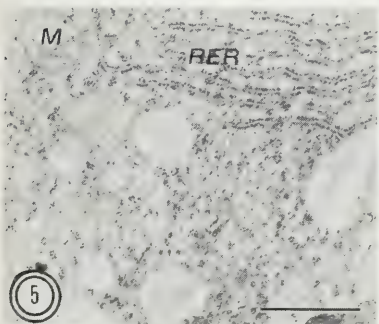
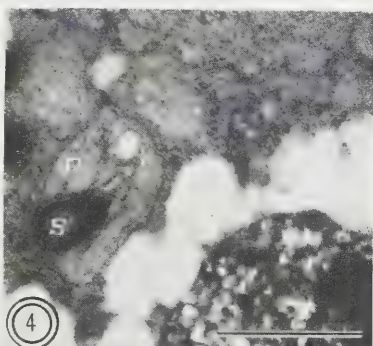
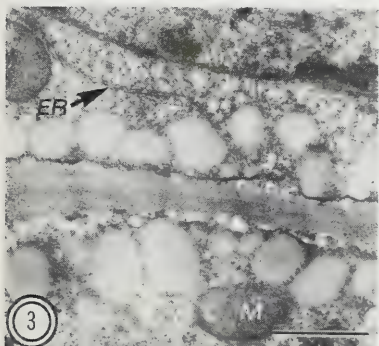
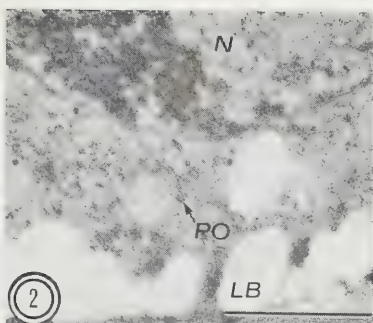
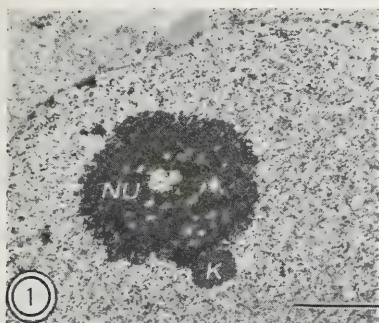


FIG. 1.

Nucleolus (NU) with a karyosome (K) in a viable root-tip cell after 3 days of incubation. X 16,000. (Bar 2.3 cms = 2 μ m).

FIG. 2.

Viable embryo cells with polysomes (PO) in the cytoplasm after 5 days of incubation. Configurations suggest the presence of mRNA. X 30,000 (Bar 3 cms = 1 μ m).

FIG. 3.

Cytoplasm in the radicle meristem after 3 days of imbibition showing fragments of ER and mitochondria with few cristae. X 6,100 (Bar 2 cms = 1 μ m).

FIG. 4.

Plastid (P) in a viable embryo with developing starch grain (S) after 10 days of incubation. Small vesicles are budding from the inner membrane. X 30,000 (Bar 3 cms = 1 μ m).

FIG. 5.

Rough ER in a meristematic cell of the root-tip of a viable embryo after 10 days of incubation. X 20,000 (Bar 2 cms = 1 μ m).

FIG. 6.

Dictyosomes (D) in a meristematic cell of the root tip of a viable embryo after 15 days of incubation. X 20,000 (Bar 2 cms = 1 μ m).

REFERENCES

- BONNETT, H. T. and NEWCOMB, E. H., 1965. Polyribosomes and cisternal accumulations in root cells of radish. *Jnl Cell Biol.* **27**: 423-431.
- BROWN, N. A. C. and VAN STADEN, J., 1973. The effect of scarification, leaching, light, stratification, oxygen and applied hormones on germination of *Protea compacta* and *Leucadendron daphnoides*. *Jl S. Afr. Bot.* **39**: 185-195.
- VAN STADEN, J., GILLILAND, M. G. and BROWN, N. A. C., 1975a. Ultrastructure of dry viable and non-viable *Protea compacta* embryos. *Ztschr. Pflanzenphysiol.* **76**: 28-35.
- VAN STADEN, J., GILLILAND, M. G., DREWES, S. E. and DAVEY, J. E., 1975b. Changes in the food reserves of viable and non-viable *Protea compacta* embryos during incubation. *Ztschr. Pflanzenphysiol.* **76**: 369-377.
- VAN STADEN, J., DAVEY, J. E. and DU PLESSIS, L. M., 1975c. Lipid utilization in viable and non-viable *Protea compacta* embryos during germination. *Ztschr. Pflanzenphysiol.* (In Press.)
- VILLIERS, T. A., 1973. Ageing and the longevity of seeds in field conditions. In: W. Heydecker, (ed.) *Seed ecology*. 265-288. London: Butterworths.